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## Review

Emerging roles of chloride channels in human diseases<sup>☆</sup>Livia Puljak, Gordan Kilic<sup>\*</sup>*Department of Internal Medicine, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-8887, USA*

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**Abstract**

In the past decade, there has been remarkable progress in understanding of the roles of  $\text{Cl}^-$  channels in the development of human diseases. Genetic studies in humans have identified mutations in the genes encoding  $\text{Cl}^-$  channels which lead to a loss of  $\text{Cl}^-$  channel activity. These mutations are responsible for the development of a variety of deleterious diseases in muscle, kidney, bone and brain including myotonia congenita, dystrophia myotonica, cystic fibrosis, osteopetrosis and epilepsy. Recent studies indicate that some diseases may develop as a result of  $\text{Cl}^-$  channel activation. There is growing evidence that the progression of glioma in the brain and the growth of the malaria parasite in red blood cells may be mediated through  $\text{Cl}^-$  channel activation. These findings suggest that  $\text{Cl}^-$  channels may be novel targets for the pharmacological treatment of a broad spectrum of diseases. This review discusses the proposed roles of abnormal  $\text{Cl}^-$  channel activity in the pathogenesis of human diseases.

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**Keywords:**  $\text{Cl}^-$  channels; Cystic fibrosis; Osteopetrosis; Epilepsy; Glioma; Malaria**1. Introduction**

Chloride channels are pore-forming membrane proteins that allow the passive transport of  $\text{Cl}^-$  across biological membranes. They are ubiquitously expressed in almost all eukaryotic cells [1]. In the plasma membrane,  $\text{Cl}^-$  channels are essential for the regulation of cell volume, the transepithelial transport of salt and water as well as the modulation of the electrical excitability in neurons.  $\text{Cl}^-$  channels are also found in membranes of intracellular organelles such as lysosomes and endosomes [2]. In these compartments,  $\text{Cl}^-$  channels play a key role in the regulation of organelle volume and pH, factors which are important for the delivery of membrane transport proteins to the plasma membrane.

$\text{Cl}^-$  channels open in a process called gating and have been functionally classified according to their gating mechanisms, depending on changes in the membrane electric potential (voltage-dependent  $\text{Cl}^-$  channels, CIC channels), activation of a

protein kinase (cystic fibrosis transmembrane conductance regulator (CFTR)  $\text{Cl}^-$  channels), increases in intracellular  $\text{Ca}^{2+}$  levels ( $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels, CaCC channels), and binding of a ligand (glycine- or  $\gamma$ -aminobutyric acid (GABA)-activated  $\text{Cl}^-$  channels). Furthermore,  $\text{Cl}^-$  channels can also be activated by an increase in cell volume, extracellular acidification, the degree of phosphorylation and the binding of ATP. Despite the variety of functional  $\text{Cl}^-$  channels, molecular biology has revealed only three well-established gene families of  $\text{Cl}^-$  channels: CIC, CFTR and ligand-gated  $\text{Cl}^-$  channels [3]. It should be noted that the CFTR belongs to the gene family of ABC transporters, which normally function as transport ATPases. CFTR is the only member which is known to function as an ion channel [3]. The discrepancy between the number of functional  $\text{Cl}^-$  channels and actual cloned proteins may be related to the fact that there are no “ $\text{Cl}^-$  channel signature” sequences conserved among the known channel families. Thus, it is likely that entire families of  $\text{Cl}^-$  channels have not yet been identified.

The role of  $\text{Cl}^-$  channels in the physiology of neurons, muscle and epithelial cells has been extensively studied [1]. Opening of  $\text{Cl}^-$  channels in the plasma membrane shifts the membrane potential towards negative values resulting in

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inhibition of the electrical excitability in neurons and muscle cells. Defective  $\text{Cl}^-$  channel activity leads to hyperexcitability and appears to be the principal cause for the development of myotonia congenita, dystrophia myotonica, hereditary hyperkplexia, epilepsy and chronic insomnia. While the most important role of  $\text{Cl}^-$  channels in neurons and muscle cells is to regulate the excitability, the primary function of these channels in epithelial cells is to mediate the transepithelial transport of salt and water. There is increasing evidence that defective  $\text{Cl}^-$  transport in epithelial cells is a critical event that leads to the development of various forms of cystic fibrosis (CF) and Bartter syndrome [2].  $\text{Cl}^-$  channels in intracellular compartments have a distinct role in maintaining pH of these compartments. Opening of  $\text{Cl}^-$  channels provides the  $\text{Cl}^-$  influx that is required for the acidification by  $\text{H}^+$ -ATPase. Numerous studies have demonstrated that a low pH is essential for the insertion of transport proteins into the plasma membrane, and the cellular uptake of hormones and vitamins [3]. Defective  $\text{Cl}^-$  channels in intracellular compartments contribute importantly to the development of osteopetrosis in the bone and Dent's disease in the kidney. Thus,  $\text{Cl}^-$  channel dysfunction may play an important role in the pathogenesis of many diseases. Genetic studies in humans have identified mutations in the genes encoding  $\text{Cl}^-$  channels that are directly responsible for the development of these diseases [2,4]. In addition, there is emerging evidence that a loss of  $\text{Cl}^-$  channel activity may also mediate the development of chronic pancreatitis, bronchiectasis, congenital bilateral aplasia of vas deferens, alcoholism, cataract and Best's disease. While the  $\text{Cl}^-$  channel proteins involved have been cloned, the cellular mechanisms linking  $\text{Cl}^-$  channel dysfunction to the pathogenesis of these diseases are still unknown.

Recent studies indicate that in addition to the inhibition of channel activity, some diseases such as glioma and malaria may

develop as a result of stimulation of  $\text{Cl}^-$  channel activity [5–8]. These findings provide strong support for the concept that abnormal  $\text{Cl}^-$  channel activity may be a novel target for the therapeutic intervention in a broad spectrum of diseases. Fig. 1 illustrates human diseases known to be associated with abnormal  $\text{Cl}^-$  channel activity.

A comprehensive description of molecular structure and the physiology of  $\text{Cl}^-$  channels is beyond the scope of this review and can be found in excellent recent reviews [1,2]. The purpose of this review is to give a short overview and discuss the proposed roles of abnormal  $\text{Cl}^-$  channel activity in a variety of human diseases.

## 2. Dysfunction of $\text{Cl}^-$ channels in the plasma membrane

### 2.1. Myotonia congenita

Myotonia congenita is a hereditary muscle disorder characterized by muscle stiffness and a delay in relaxation after a voluntary muscle contraction. There are two forms of myotonia congenita: autosomal-recessive Becker disease and autosomal-dominant Thomsen disease. Both disorders are caused by mutations in the gene *CLCN1* that encodes a subunit of the skeletal muscle  $\text{Cl}^-$  channel CIC-1 (Table 1). The molecular mechanism involved in the pathogenesis of myotonia congenita is well understood [9]. The  $\text{Cl}^-$  conductance mediated through activation of CIC-1 channels is severely reduced in patients with myotonia congenita [10,11]. Because the  $\text{Cl}^-$  conductance contributes significantly to the resting membrane conductance and helps to inhibit electrical activity, a loss of CIC-1 channel activity leads to muscle hyperexcitability and anomalous firing of action potentials. To date, more than 80 different mutations in *CLCN1* gene have been identified in myotonia congenita, and most of them result in a recessive form of the disease [9,12].

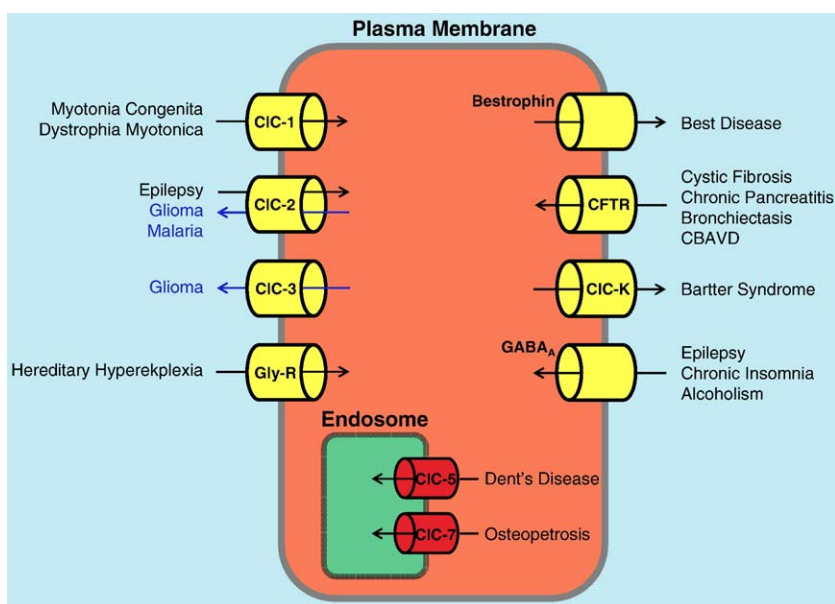


Fig. 1.  $\text{Cl}^-$  channels and human diseases. A cartoon shows the cellular locations, the type of  $\text{Cl}^-$  channel and diseases known to be associated with a downregulation of  $\text{Cl}^-$  channel activity. Arrows indicate the direction of  $\text{Cl}^-$  transport under normal physiologic conditions. In glioma and malaria,  $\text{Cl}^-$  channel activity is upregulated (blue arrows).

Table 1  
Cl<sup>−</sup> channelopathies

Cl <sup>−</sup> channel	Human disease	Reference
ClC-1	Myotonia congenita Becker	[10]
	Myotonia congenita Thomsen	[102]
	Dystrophia myotonica 1	[18,20]
	Dystrophia myotonica 2	[18,19]
ClC-2	Childhood absence epilepsy type 3	[103]
	Juvenile absence epilepsy	[103]
	Juvenile myoclonic epilepsy	[103]
	Epilepsy with grand mal seizures on awakening	[103]
ClC-Kb	Barter Syndrome III	[104]
ClC-Ka, ClC-Kb (Barttin)	Barter Syndrome IV or BSND	[76,77]
ClC-5	Dent's disease	[105–107]
ClC-7	Autosomal dominant osteopetrosis	[83]
	Autosomal recessive osteopetrosis	[82]
CFTR	Cystic fibrosis	[108]
	Idiopathic chronic pancreatitis	[109–111]
	Bronchiectasis	[35,112–114]
	Congenital bilateral absence of vas deferens	[40,115–117]
Bestrophin	Best's disease	[69,118,119]
	Adult-onset vitelliform macular dystrophy	[120]
	Concentric annular macular dystrophy	[120]
	Cataract	[57–60]
Unknown GABA <sub>A</sub>	Juvenile myoclonic epilepsy	[121]
	Childhood absence epilepsy type 2	[122]
	Generalized epilepsy with febrile seizures plus	[123]
	Severe myoclonic epilepsy in infancy	[124]
	Alcoholism	[125,126]
	Insomnia	[49]
GlyR	Hereditary hyperekplexia	[41,44,127,128]

Cl<sup>−</sup> channel proteins and human diseases associated with mutations in these proteins.

## 2.2. Dystrophia myotonica

Dystrophia myotonica (DM) is an autosomal-dominant inherited disease that primarily affects muscles and the nervous system, causing myotonia, heart block and cataract [13]. Similar to myotonia congenita, muscles in patients with DM have the ability to contract but have decreasing power to relax. The most important difference between DM and myotonia congenita is that patients with DM develop a severe muscle dystrophy and weakness. Two genetic forms of dystrophia myotonica have been described: DM1 and DM2 (Table 1). Genetic studies firmly established that the functional defects in DM1 and DM2 are caused by DNA expansion mutations [14–17]. These DNA repeats do not encode any portion of the protein, but when transcribed, cause the mRNA to be retained in the nucleus [18]. Recent studies have demonstrated that nuclear accumulation of mRNA leads to an irregular splicing of a ClC-1 pre-mRNA [19–21]. This may cause inhibition of the Cl<sup>−</sup> conductance as observed in patients with DM [22]. Thus, a loss of ClC-1 channel activity due to aberrant pattern of ClC-1 splicing appears to be the dominant mechanism responsible for the

development of myotonia in both forms of dystrophia myotonica [23].

## 2.3. Cystic fibrosis

Cystic fibrosis (CF) is the most common autosomal-recessive disease in Caucasians that leads to early death. Mucous secretions of CF patients are abnormally viscid leading to the obstruction of the luminal space and recurrent cycles of inflammation and fibrosis, which ultimately destroy the affected organs. The disease is caused by mutations in the *CFTR* gene (Table 1). This gene encodes a Cl<sup>−</sup> channel that is activated by increases in intracellular cAMP levels [24]. CFTR is primarily localized at the apical surface of epithelial cells lining the airway, gut, exocrine glands, where it regulates the transepithelial salt and water transport [25]. Although the physiology of CFTR is complex, it appears that a loss in CFTR activity is a primary event which is responsible for defective Cl<sup>−</sup> uptake into the epithelial cells and the development of CF symptoms [26]. Two models have been proposed to explain defective salt transport in CF patients [27]. In one model, diminished Cl<sup>−</sup> reabsorption through CFTR causes a decrease in Na<sup>+</sup> uptake through the epithelial Na<sup>+</sup> channels (ENaC) in the plasma membrane resulting in an increased salt content in the luminal space. In this high-salt model, elevated salt concentration may ultimately lead to a severe obstruction of secretory functions in epithelial cells. In the alternative model, the increased Na<sup>+</sup> absorption through ENaC mediates an increase in Cl<sup>−</sup> reabsorption through non-CFTR dependent Cl<sup>−</sup> absorptive pathways, which leads to dehydration of airway surfaces and defective mucociliary transport. This model is supported by the recent studies in which mice with airway-specific overexpression of ENaC develop a severe lung disease similar to CF [28]. Regardless of which model of NaCl reabsorption is used to explain the pulmonary deficiency in CF, there is a strong consensus that patients with the disease lack the normal ability to transport Cl<sup>−</sup> through CFTR in the airways.

More than 1000 mutations of *CFTR* gene have been reported so far [29]. Some *CFTR* mutations have also been linked to other diseases, such as chronic pancreatitis, congenital bilateral absence of vas deferens, bronchiectasis, asthma, aspergillosis and sinusitis [30]. These findings have led to the hypothesis that inhibition of CFTR activity may play an important role in the pathogenesis of many diseases in epithelial tissue.

## 2.4. Chronic pancreatitis

Pancreatitis is inflammation of the pancreas caused by digestive enzymes that autodigest the pancreas itself. Several groups have reported an increased prevalence of *CFTR* mutations in patients with idiopathic chronic pancreatitis (Table 1). While up to 30% of the patients with chronic pancreatitis were found to have at least one *CFTR* mutation, patients with two or more *CFTR* mutations appear to be at higher risk [31,32].

Although the *CFTR* mutations may be involved in the progression of chronic pancreatitis, the cellular mechanisms

responsible for inflammation of the pancreas are not known. It has been proposed that an inhibition of  $\text{Cl}^-$  channel activity caused by the *CFTR* mutations leads to a decrease in the ductal pH. Subsequently, the ductal acidification may promote a defective solubilization of proteins, inhibition of zymogen granule trafficking and stimulation of the pancreatic enzyme, trypsinogen. This concept is further supported with the finding that even a moderate reduction in *CFTR* activity in a heterozygous state increases the risk for chronic pancreatitis [30].

### 2.5. Bronchiectasis

Bronchiectasis is characterized by an abnormal irreversible dilatation of bronchi and represents the end stage of a variety of pathological processes that cause the destruction of the bronchial wall and supporting tissues [33]. *CFTR* is expressed in several cell types of the airway and mutations in the *CFTR* gene have been found in several lung disorders [34].

Several lines of evidence suggest that mutations in the *CFTR* gene may also contribute to the pathogenesis of bronchiectasis (Table 1). In addition to defective *CFTR* activity, the environmental factors and other genes may also be involved in the development of this disease [35]. In contrast to these findings, other studies indicate that the frequency of *CFTR* mutations in patients with bronchiectasis was not significantly higher than in general population [36,37]. These studies were done with a small number of patients and imply that further studies in a larger cohort of clinically well-defined patients are needed. Thus, it is still unknown whether defective *CFTR* activity in lung is involved in the pathogenesis of bronchiectasis.

### 2.6. Congenital bilateral aplasia of vas deferens

Congenital bilateral aplasia of the vas deferens (CBAVD) leads to male infertility. This disease is also characterized by the presence of subclinical cystic fibrosis symptoms including elevated sweat  $\text{Cl}^-$  concentrations and sinusitis [38]. Furthermore, because the vas deferens is absent in most of male patients with cystic fibrosis, CBAVD emerged as a primary genital form of cystic fibrosis [39].

Mutations and the splice variants in the *CFTR* gene are found in the majority of CBAVD patients (Table 1). While patients with cystic fibrosis have mutations in both copies of the *CFTR* gene, most patients with CBAVD have mutations in only one copy of the gene [40]. Although CBAVD appears to be closely associated with a loss of the *CFTR* activity, it is not known how and by which mechanisms,  $\text{Cl}^-$  channel dysfunction leads to an early regression of the mesonephric duct and progressive male infertility.

### 2.7. Hereditary hyperekplexia

Hyperekplexia, also known as hereditary startle disease, is characterized by an exaggerated startle reflex, neonatal hypertonia, nocturnal myoclonus and a chronic accumulation of injuries caused by startle-induced falls. Both autosomal-dominant and autosomal-recessive hyperekplexia are caused by

mutation in the gene *GLRA1* encoding the  $\alpha$ -1 subunit of the glycine receptor (Table 1). Mutations in the gene *GLRB* encoding the  $\beta$ -subunit of the glycine receptor can also lead to the development of autosomal-recessive hyperekplexia [41,42].

The role of glycine-activated  $\text{Cl}^-$  channels in the pathogenesis of hyperekplexia is well defined. Stimulation of  $\text{Cl}^-$  conductance by glycine facilitates fast inhibitory neurotransmission in the brainstem and spinal cord, resulting in the reduction of excitatory startle responses [43]. Mutations in both subunits inhibit the binding of glycine to its receptor and reduce the magnitude of the  $\text{Cl}^-$  conductance [44]. Similar to myotonia congenita and dystrophia myotonica, the reduction in  $\text{Cl}^-$  conductance leads to hyperexcitability of the pontomedullary reticular neurons and abnormal spinal reciprocal inhibition.

### 2.8. Epilepsy

Epilepsy is a recurrent transient disturbance of brain function resulting from hyperexcitability of neurons and is unrelated to infection or acute cerebral insult. Idiopathic epilepsy accounts for about 40% of the cases. Recent studies have provided evidence that heterozygous mutations in a gene encoding for  $\text{Cl}^-$  channel *ClC-2* (*CLCN2*) may be responsible for several subtypes of idiopathic generalized epilepsy including childhood absence epilepsy type 3, juvenile absence epilepsy, juvenile myoclonic epilepsy and epilepsy with grand mal seizures on awakening (Table 1). Other studies found no correlation between mutations in the *CLCN2* gene and epilepsy [45]. Thus, the role of *ClC-2* in the pathogenesis of epilepsy is not known for certain. In contrast to *ClC-2*, the evidence for the involvement of synaptic  $\text{GABA}_A$ -receptor  $\text{Cl}^-$  channels in the pathogenesis of certain forms of epilepsies is firmly established [46]. Specifically, mutations in *GABRG2*, the gene encoding  $\text{GABA}_A$   $\gamma_2$ -subunit, mediate several different types of epilepsies including juvenile myoclonic epilepsy, childhood absence epilepsy 2, generalized epilepsy with febrile seizures plus and its severe form of myoclonic epilepsy in infancy (Table 1).

The physiologic mechanisms responsible for the development of epilepsy are well established. In the healthy brain, physiologic stimulation of  $\text{Cl}^-$  influx through  $\text{Cl}^-$  channels provides a mechanism for tonic inhibition of the electrical activity in neurons. Consequently, dysfunction of either the *ClC-2* or the  $\text{GABA}_A$   $\text{Cl}^-$  channel activity may make neurons more susceptible for excitation, and subsequently may induce epilepsy. This model is further supported by the findings that drugs used to treat epilepsy (i.e., barbiturates and benzodiazepines, vigabatrin and tiagabine) function through stimulation of  $\text{GABA}$ -mediated  $\text{Cl}^-$  channels [47]. Conversely, drugs that decrease the concentration of  $\text{GABA}$  in the brain by inhibiting the synthesis of  $\text{GABA}$  cause seizures [48].

It should be noted that  $\text{GABA}_A$ -receptors are composed from the repertoire of 19 subunit variants and are widespread throughout the brain. Thus, it is not surprising that a close correlation exists between various subunit gene polymorphisms and a broad spectrum of neuropsychiatric disorders, including alcoholism, schizophrenia, anxiety, and bipolar affective



disorders [47]. Consequently, future studies of new mutations in animal models may provide more selective targets for the treatment of neuropsychiatric disorders.

### 2.9. Chronic insomnia

Genetic studies have identified a missense mutation in the gene encoding the  $\beta$ -3 subunit of the GABA<sub>A</sub> receptor (*GABRB3*) in a patient with chronic insomnia who had a family history of sleep problems [49] (Table 1). The patient was found to be heterozygous for this mutation. Functional analysis of this mutation revealed that the Cl<sup>−</sup> conductance evoked by GABA decays faster in mutated channels than in wild-type channels. These results indicate that mutations in the GABA<sub>A</sub>-receptors may be involved in the pathogenesis of insomnia.

Consistent with these results, deletion of *GABRB3* gene inhibited the hypnotic response to oleamide in mice [50]. Furthermore, sleep disorders including insomnia have been effectively treated with the therapeutic agents that stimulate GABA<sub>A</sub>-receptor Cl<sup>−</sup> channel activity [47]. These findings are consistent with the hypothesis that a loss of GABA-mediated Cl<sup>−</sup> channel activity may be directly responsible for abnormal electrical activity of neurons and the development of insomnia. The final confirmation of this hypothesis will require further studies.

### 2.10. Alcoholism

Multiple lines of evidence suggest that GABA<sub>A</sub>-receptor Cl<sup>−</sup> channels are involved in many of the neurochemical pathways contributing to alcohol use [51]. GABA<sub>A</sub>-receptor agonists tend to potentiate the behavioral effects of alcohol, while GABA<sub>A</sub>-receptor antagonists attenuate these effects. Furthermore, GABA<sub>A</sub>-receptors have also been implicated in ethanol tolerance and dependence [51]. The precise mechanism by which GABA receptors mediate these effects of ethanol are still unknown.

Recent studies found a close correlation between the susceptibility for alcohol dependence and *GABRA2*, a gene encoding the  $\alpha$ -2 subunit of GABA<sub>A</sub> receptor (Table 1). Other studies found no such correlation [52–54]. Similar to *GABRA2*, the evidence for and against the involvement of GABA-mediated inhibition in the susceptibility for alcohol dependence, have also been obtained for *GABRA6*, another gene that encodes the  $\alpha$ -6 subunit of GABA<sub>A</sub> receptor [54, 55]. Consequently, it is still unknown whether defective GABA-mediated Cl<sup>−</sup> channel activity plays the dominant role in the susceptibility for alcohol dependence.

### 2.11. Cataract

A cataract is a cloudy area in the lens of the eye that leads to impaired vision. Studies in animals have clearly demonstrated that the lens swelling precedes opacification [56]. Consequently, the ability of lens cells to maintain cell volume within narrow physiologic limits is critical for the lens to remain transparent. Stimulation of volume-activated Cl<sup>−</sup>

channels in response to cell swelling plays a key role in the regulation of cell volume. Several recent studies indicate that inhibition of volume-activated Cl<sup>−</sup> channels by tamoxifen or Cl<sup>−</sup> channel blockers in animals, causes the lens to swell and opacify [57–60]. These results are consistent with the hypothesis that reduced activity of volume-activated Cl<sup>−</sup> channels in the plasma membrane of the lens cells may be involved in the development of cataract.

In humans, there are reports that tamoxifen, an antiestrogen frequently used in the treatment of breast cancer, increases the risk of cataract after prolonged use [61–63]. However, in other studies, no such risk is observed [64–66]. These findings suggest that the role of volume-activated Cl<sup>−</sup> channels in the pathogenesis of cataract in humans still needs to be defined.

### 2.12. Best's disease

Best's disease or juvenile-onset vitelliform macular dystrophy, is an autosomal-dominant disease of the central retina characterized by macular lesions resembling the yolk of an egg ('vitelliform'). This disease is caused by mutations in the *VMD2* gene that encodes bestrophin, a transmembrane protein with putative Ca<sup>2+</sup>-dependent Cl<sup>−</sup> channel activity in the retinal pigment epithelium [67, 68] (Table 1). Mutations in the *VMD2* gene also appear to be involved in an adult-onset vitelliform macular dystrophy and concentric annular macular dystrophy (Table 1).

The cellular mechanisms responsible for the retinal degeneration in Best's disease are still poorly understood. Recent studies have demonstrated that mutated bestrophin forms a defective plasma membrane Ca<sup>2+</sup>-dependent Cl<sup>−</sup> channel [69]. Several lines of evidence suggest that Cl<sup>−</sup> channel activity mediated by the bestrophins may have multiple functions, both at the plasma membrane and in intracellular organelles. In the plasma membrane of the retinal pigment epithelium, Cl<sup>−</sup> channels regulate the composition of the extracellular fluid as well as cell volume. Defects in Cl<sup>−</sup> channel activity in the plasma membrane may result in retinal degeneration as a consequence of abnormal regulation of cell volume and/or the extracellular fluid composition [70]. It is proposed that plasma membrane transport by the retinal pigment epithelium helps maintain the composition of this fluid within a range that supports phototransduction [71]. Other studies are consistent with the concept that bestrophins regulate the ionic environment of inside organelles in the endosomal-lysosomal pathway, and retinal degeneration may result from defects in lysosomal trafficking or function [70].

### 2.13. Bartter syndrome

Bartter syndrome is a genetically heterogeneous disorder caused by defective renal tubular electrolyte transport [72,73]. Five genotypes of Bartter's syndrome have been described so far, but only Bartter types III and IV are caused by defective activity of ClC-Ka and ClC-Kb channels. Both Cl<sup>−</sup> channels are members of the ClC family, and they both need barttin for the functional expression in kidney cells. Barttin is a small

accessory  $\beta$ -subunit, and is normally present in the complexes with CIC-Ka and CIC-Kb.

Bartter syndrome type III or classic Bartter syndrome, is caused by mutations in the *CLCNKB*, human gene encoding CIC-Kb (Table 1). Typically, the onset of the syndrome occurs during infancy or childhood, with a characteristic set of metabolic abnormalities including hypokalemia, metabolic alkalosis, hyperreninemia and hyperaldosteronism. The disease develops as a result of decreased renal  $\text{Cl}^-$  transport that leads to a reduction of NaCl reabsorption from urine. A large amount of NaCl and water are lost in urine resulting in stimulation of secretion of renin, angiotensin II and aldosterone [74]. Subsequent increase in the levels of these hormones is directly responsible for the symptoms in Bartter III syndrome.

Bartter IV syndrome is caused by a mutation in barttin (Table 1). A lack of functional barttin causes inhibition of  $\text{Cl}^-$  channel activity in both CIC-Ka and CIC-Kb [75]. Furthermore, because both channels are expressed in kidney and the inner ear, the symptoms of Bartter IV syndrome are severe [76]. The disease is characterized by congenital deafness, increased urine flow, excessive thirst and a failure to thrive. This syndrome is also known as Bartter syndrome with sensorineural deafness (BSND) [77]. Although, the physiology of hearing is not completely understood, it appears that  $\text{Cl}^-$  channels may provide an efficient mechanism for  $\text{Cl}^-$  recycling at the basolateral membrane of stria vascularis in the inner ear. This recycling is important for homeostasis of  $\text{K}^+$  ions in the inner ear fluid, which is essential for proper hearing [75].

### 3. Dysfunction of $\text{Cl}^-$ channels in intracellular membranes

#### 3.1. Osteopetrosis

Osteopetrosis is a disorder characterized by dense and fragile bones devoid of bone marrow [78]. Genetic studies have identified mutations in human *CLCN7* gene that encodes the CIC-7 channel, which can induce different forms of osteopetrosis (Table 1). There are two forms of osteopetrosis. Autosomal-dominant osteopetrosis is characterized by sclerosis, predominantly involving the spine, the pelvis and the skull base [79–81]. An autosomal-recessive mutation leads to severe and intermediate osteopetrosis [82]. Homozygous mutations lead to malignant infantile osteopetrosis [82], while patients with heterozygous, dominant mutations suffer from a less severe form [83].

The CIC-7 channel resides in membranes of the late endosomes and lysosomes in osteoclasts. Osteoclasts play a key role in the bone resorption during adult life [84]. These cells degrade the bone mineral through acidification. The bone degradation is mediated through exocytosis of a large number of intracellular vesicles that fuse with bone-facing plasma membrane to form a ruffled border membrane, which is the actual resorbing organelle [85]. Activation of CIC-7 channels in intracellular vesicles of healthy subjects provides the  $\text{Cl}^-$  conductance required for efficient proton pumping by the  $\text{H}^+$ -ATPase [82]. Exocytic insertion of  $\text{H}^+$ -ATPase and CIC-7 into the ruffled border membrane results in acidification of the

resorption lacuna which promotes the dissolution of the mineral phase and the enzymatic degradation of the organic bone matrix. A loss of the CIC-7 channel activity leads to a defect in bone degradation and the development of osteopetrosis [86].

#### 3.2. Dent's disease

Dent's disease, also known as X-linked recessive nephrolithiasis, is an inherited disorder caused by mutations in the gene *CLCN5* that encodes the  $\text{Cl}^-$  channel CIC-5 [87] (Table 1). This disease is characterized by low-molecular weight proteinuria, hyperphosphaturia and hypercalciuria, which leads to the formation of kidney stones [88]. The CIC-5 channel resides in the endosomal membrane and plays a key role in the acidification of these compartments in proximal renal tubules. Recent studies indicate that many CIC channels including CIC-5 which are similar to the bacterial homolog CIC-e1, may also function as a  $\text{Cl}^-/\text{H}^+$  exchange transporter [89]. In any case, it appears that the influx of  $\text{Cl}^-$  into the endosomal compartment through CIC-5 provides the negative ion which is essential for electrical neutralization of the proton influx generated either by  $\text{H}^+$ -ATPase or CIC-5 itself [87].

There is emerging evidence that a loss of CIC-5 channel activity inhibits endocytosis of parathyroid hormone and vitamin D as well as receptor-mediated and fluid-phase endocytosis. Over time these changes result in a severe loss of proteins and the formation of kidney stones [90]. Genetic studies have shown that the elimination of CIC-5 in knock-out mice reproduced the low-molecular weight proteinuria observed in patients with Dent's disease [91]. These findings have firmly established that a loss of CIC-5 channel activity plays a key role in the pathogenesis of Dent's disease.

### 4. Stimulation of $\text{Cl}^-$ channel activity

#### 4.1. Glioma

Gliomas are a heterogeneous group of primary brain tumors derived from glial cells. These cells exhibit uncontrolled proliferation, and have the ability to disperse from the tumor site and invade the healthy brain tissue [92]. Glioma cells squeeze through narrow extracellular brain spaces by rapidly changing cell volume. Notably, these changes are inhibited by  $\text{Cl}^-$  channel blockers that render glioma cells unable to invade [5,93]. Glioma cells also have the capacity to thrive in the vicinity of an edematous environment. Under conditions of the decreased osmolarity in extracellular media, glioma cells can rapidly regulate their volume back to the baseline levels through stimulation of plasma membrane  $\text{Cl}^-$  channels. Thus, it appears that the functional interactions between changes in cell volume and volume-activated  $\text{Cl}^-$  channels in glioma cells is important for proliferation and the growth of glioma in the brain.

Recent studies indicate that CIC-2 and CIC-3 channels may contribute to the ability of glioma cells to invade the healthy brain [6]. However, many studies have demonstrated that CIC-2 and CIC-3 are no longer the molecular candidates for volume-regulated  $\text{Cl}^-$  channels [4]. Thus, the molecular identity of  $\text{Cl}^-$

channels involved in the pathogenesis of glioma still needs to be defined.

#### 4.2. Malaria

The malaria parasite (*Plasmodium falciparum*) in the course of its complex life cycle invades red blood cells (RBCs) of its vertebrate host [94]. This intra-erythrocytic phase of the parasite life cycle is responsible for all symptoms of malaria, a disease that causes close to five billion episodes of clinical illness and up to three million deaths each year [95,96].

The cellular mechanisms responsible for the growth of the malaria parasite are poorly understood [97]. The malaria parasite remodels RBCs to provide nutrients for its own needs [94]. There is emerging evidence that stimulation of  $\text{Cl}^-$  channel activity in the plasma membrane of RBCs contributes importantly to the growth of the malaria parasite [7,8,98,99]. Two models have been proposed to explain the growth of malaria parasite. According to the first model, infection with the malaria parasite upregulates endogenous  $\text{Cl}^-$  channels in the RBCs which promotes the growth of the parasite [99]. Recent studies have demonstrated that infection with the malaria parasite leads to upregulation of endogenous  $\text{ClC-2}$  channel. Surprisingly, a loss of  $\text{ClC-2}$  channel activity in  $\text{ClC-2}$  knockout mice had no effect on intraerythrocytic parasite survival [100]. Similarly, a loss of CFTR activity in the erythrocytes from CF patients had no effect on growth of the malaria parasite in vitro [101]. Thus, the role of endogenous RBC's  $\text{Cl}^-$  channels in the progression of malaria is still unknown. In the second model, a parasite-encoded  $\text{Cl}^-$  channel may contribute to the growth of the malaria parasite. Recent genetic studies have identified a novel gene in the parasite genome which encodes a distinct  $\text{Cl}^-$  channel, named plasmodium surface anion channel (PSAC) [7, 98]. Upon infection, PSAC is expressed and inserted into the plasma membrane, resulting in stimulation of  $\text{Cl}^-$  channel activity in the infected RBCs [7].

#### 5. Conclusions and perspectives

It is well documented that mutations in the genes encoding  $\text{Cl}^-$  channels contribute importantly to the pathogenesis of a broad spectrum of diseases. A loss of  $\text{Cl}^-$  channel activity in the plasma membrane is linked to the development of diseases associated with defective transepithelial salt transport, muscle and neuronal excitability. Dysfunction of  $\text{Cl}^-$  channels in the intracellular compartments leads to osteopetrosis and Dent's disease. Although a loss of  $\text{Cl}^-$  channel activity appears to be the underlying mechanism for the progression of many diseases, some diseases such as glioma and malaria may develop as a result of stimulation of  $\text{Cl}^-$  channel activity.

With the application of recently developed biophysical techniques and gene technologies to study the physiologic functions of ion channels in greater detail, there has been remarkable progress in understanding of the roles of  $\text{Cl}^-$  channels in the pathogenesis of human diseases [4]. Consequently,  $\text{Cl}^-$  channels are emerging as potential pharmacological targets for the treatment of many diseases. Despite this

progress, precise biological functions of  $\text{Cl}^-$  channels in the physiology of different organs are still poorly understood. Thus, future studies aimed to define these functions may open new avenues for developing novel therapies for the treatment of human diseases.

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